

WHAT IS CLAIMED IS:

1 1. An isolated infectious chimeric respiratory
2 syncytial virus (RSV) comprising a major nucleocapsid (N)
3 protein, a nucleocapsid phosphoprotein (P), a large polymerase
4 protein (L), a RNA polymerase elongation factor, and a partial
5 or complete RSV genome or antigenome of one RSV strain or
6 subgroup virus combined with a heterologous gene or gene
7 segment of a different RSV strain or subgroup virus to form a
8 chimeric RSV genome or antigenome.

1 2. The chimeric RSV of claim 1, wherein the
2 chimeric genome or antigenome comprises a partial or complete
3 human RSV genome or antigenome of one RSV subgroup or strain
4 combined with a heterologous gene or gene segment from a
5 different, human or non-human RSV subgroup or strain.

1 3. The chimeric RSV of claim 2, wherein the
2 heterologous gene or gene segment is from a human RSV subgroup
3 A, human RSV subgroup B, bovine RSV or murine RSV.

1 4. The chimeric RSV of claim 1, wherein the
2 heterologous gene or gene segment is selected from a NS1, NS2,
3 N, P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene or gene
4 segment.

1 5. The chimeric RSV of claim 4, wherein the
2 heterologous gene or gene segment encodes a RSV F, G or SH
3 glycoprotein or a cytoplasmic domain, transmembrane domain,
4 ectodomain or immunogenic epitope thereof.

1 6. The chimeric RSV of claim 1, wherein the
2 chimeric genome or antigenome comprises a partial or complete
3 human RSV A subgroup genome or antigenome combined with a
4 heterologous gene or gene segment from a human RSV B subgroup
5 virus.

1 7. The chimeric RSV of claim 6, wherein the
2 heterologous gene or gene segment from human RSV B encodes a

1 RSV F, G or SH glycoprotein or a cytoplasmic domain,
2 transmembrane domain, ectodomain or immunogenic epitope
3 thereof.

1 8. The chimeric RSV of claim 6, wherein one or more
2 human RSV B subgroup glycoprotein genes F, G and SH or a
3 cytoplasmic domain, transmembrane domain, ectodomain or
4 immunogenic epitope thereof is substituted within a RSV A
5 genome or antigenome.

1 9. The chimeric RSV of claim 8, wherein one or both
2 human RSV B subgroup glycoprotein genes F and G is substituted
3 to replace one or both counterpart F and G glycoprotein genes
4 in the RSV A genome or antigenome.

1 10. The chimeric RSV of claim 9, wherein both human
2 RSV B subgroup glycoprotein genes F and G are substituted to
3 replace the counterpart F and G glycoprotein genes in the RSV
4 A genome or antigenome.

1 11. The chimeric RSV of claim 1, wherein a first
2 heterologous gene or gene segment is substituted to replace a
3 counterpart gene or gene segment within the partial or
4 complete RSV genome or antigenome, and a second heterologous
5 gene or gene segment is added to the partial or complete RSV
6 genome or antigenome to form the chimeric RSV genome or
7 antigenome.

1 12. The chimeric RSV of claim 1, wherein the
2 chimeric genome or antigenome is further modified by one or
3 more attenuating mutations.

1 13. The chimeric RSV of claim 12, wherein the
2 chimeric genome or antigenome incorporates at least one and up
3 to a full complement of attenuating mutations present within a
4 panel of biologically derived mutant RSV strains, said panel
5 comprising *cpts* RSV 248 (ATCC VR 2450), *cpts* RSV 248/404 (ATCC
6 VR 2454), *cpts* RSV 248/955 (ATCC VR 2453), *cpts* RSV 530 (ATCC

1 VR 2452), *cpts* RSV 530/1009 (ATCC VR 2451), *cpts* RSV 530/1030
2 (ATCC VR 2455), RSV B-1 *cp*52/2B5 (ATCC VR 2542), and RSV B-1
3 *cp*-23 (ATCC VR 2579).

1 14. The chimeric RSV of claim 12, wherein the
2 chimeric genome or antigenome incorporates at least one and up
3 to a full complement of attenuating mutations specifying a
4 temperature-sensitive amino acid substitution at Phe₅₂₁, Gln₈₃₁,
5 Met₁₁₆₉ or Tyr₁₃₂₁ in the RSV polymerase gene L, or a
6 temperature- sensitive nucleotide substitution in the gene-
7 start sequence of gene M2.

1 15. The chimeric RSV of claim 12, wherein the
2 chimeric genome or antigenome incorporates at least one and up
3 to a full complement of mutations from cold-passaged
4 attenuated RSV, said complement of mutations including
5 mutations specifying an amino acid substitution at Val₂₆₇ in
6 the RSV N gene, Glu₂₁₈ or Thr₅₂₃ in the RSV F gene, Cys₃₁₉ or
7 His₁₆₉₀ in the RSV polymerase gene L.

1 16. The chimeric RSV of claim 1, wherein each of
2 the human RSV B subgroup glycoprotein genes F and G is added
3 or substituted within a human RSV A genome or antigenome to
4 form the chimeric genome or antigenome, which is further
5 modified to incorporate one or more attenuating mutations.

1 17. The chimeric RSV of claim 16, wherein both
2 human RSV B subgroup glycoprotein genes F and G are
3 substituted to replace counterpart F and G glycoprotein genes
4 within an RSV A genome or antigenome to form the chimeric
5 genome or antigenome, which is further modified to incorporate
6 attenuating point mutations selected from (i) a panel of
7 mutations specifying temperature-sensitive amino acid
8 substitutions at Gln₈₃₁ and Tyr₁₃₂₁ in the RSV polymerase gene
9 L; (ii) a temperature-sensitive nucleotide substitution in the
10 gene-start sequence of gene M2; (iii) an attenuating panel of
11 mutations adopted from cold-passaged RSV specifying amino acid
12 substitutions Val₂₆₇ Ile in the RSV N gene, and Cys₃₁₉ to Tyr

13 and His₁₆₉₀ Tyr in the RSV polymerase gene L; or (iv) a
14 deletion of the SH gene.

1 18. The chimeric RSV of claim 12, wherein the
2 chimeric genome or antigenome incorporates at least two
3 attenuating mutations.

1 19. The chimeric RSV of claim 18, wherein the
2 chimeric genome or antigenome incorporates attenuating
3 mutations adopted from different biologically derived mutant
4 RSV strains.

1 20. The chimeric RSV of claim 12, wherein the
2 chimeric genome or antigenome includes at least one
3 attenuating mutation stabilized by multiple nucleotide changes
4 in a codon specifying the mutation.

1 21. The chimeric RSV of claim 1, formulated in a
2 dose of 10^3 to 10^6 PFU of attenuated virus.

1 22. The chimeric RSV of claim 1 further comprising
2 a nucleotide modification specifying a phenotypic change
3 selected from a change in growth characteristics, attenuation,
4 temperature-sensitivity, cold-adaptation, plaque size, host-
5 range restriction, or a change in immunogenicity.

1 23. The chimeric RSV of claim 22, wherein a SH,
2 NS1, NS2, M2ORF2, or G gene is modified.

1 24. The chimeric RSV of claim 23, wherein the SH,
2 NS1, NS2, M2ORF2, or G gene is deleted in whole or in part or
3 expression of the gene is ablated by introduction of one or
4 more stop codons in an open reading frame of the gene.

1 25. The chimeric RSV of claim 22, wherein the
2 nucleotide modification comprises a nucleotide deletion,
3 insertion, substitution, addition or rearrangement of a

4 cis-acting regulatory sequence of a selected RSV gene within
5 the chimeric RSV genome or antigenome.

1 26. The chimeric RSV of claim 25, wherein the
2 cis-acting regulatory sequence of the selected RSV gene is
3 changed to correspond to a heterologous regulatory sequence
4 comprising a counterpart cis-acting regulatory sequence of the
5 selected RSV gene from a different RSV subgroup or strain or a
6 cis-acting regulatory sequence of a different RSV gene.

1 27. The chimeric RSV of claim 25, wherein a gene
2 end (GE) signal of the NS1 or NS2 gene is modified to
3 correspond to the GE signal of the RSV N gene.

1 28. The chimeric RSV of claim 22, wherein the
2 nucleotide modification comprises an insertion, deletion,
3 substitution, or rearrangement of a translational start site
4 within the chimeric genome or antigenome.

1 29. The chimeric RSV of claim 28, wherein the
2 translational start site for a secreted form of the RSV G
3 glycoprotein is ablated.

1 30. The chimeric RSV of claim 22, wherein the
2 chimeric genome or antigenome is modified to encode a non-RSV
3 molecule selected from a cytokine, a T-helper epitope, a
4 restriction site marker, or a protein of a microbial pathogen
5 capable of eliciting a protective immune response in a
6 mammalian host.

1 31. The chimeric RSV of claim 22, which
2 incorporates a gene or gene segment from parainfluenza virus
3 (PIV) .

1 32. The chimeric RSV of claim 31, wherein the gene
2 or gene segment encodes a PIV HN or F glycoprotein.

1 33. The chimeric RSV of claim 32, wherein the gene
2 segment encodes a cytoplasmic tail, transmembrane domain,
3 ectodomain or immunogenic epitope of HN or F of PIV1, PIV2, or
4 PIV3.

1 34. The chimeric RSV of claim 1, wherein the
2 chimeric genome or antigenome comprises a partial or complete
3 human RSV genome or antigenome combined with an attenuating,
4 heterologous gene or gene segment from a bovine or murine RSV.

1 35. The chimeric RSV of claim 1 which is a virus.

1 36. The chimeric RSV of claim 1 which is a subviral
2 particle.

1 37. A method for stimulating the immune system of
2 an individual to induce protection against RSV which comprises
3 administering to the individual an immunologically sufficient
4 amount of the chimeric RSV of claim 1 combined with a
5 physiologically acceptable carrier.

1 38. The method of claim 37, wherein the chimeric
2 RSV is administered in a dose of 10^3 to 10^6 PFU.

1 39. The method of claim 37, wherein the chimeric
2 RSV is administered to the upper respiratory tract.

1 40. The method of claim 37, wherein the chimeric
2 RSV is administered by spray, droplet or aerosol.

1 41. The method of claim 37, wherein the chimeric
2 RSV is administered to an individual seronegative for
3 antibodies to RSV or possessing transplacentally acquired
4 maternal antibodies to RSV.

1 42. The method of claim 37, wherein the chimeric
2 RSV is a chimera of human RSV A and RSV B which elicits an
3 immune response against either human RSV A or RSV B.

1 43. The method of claim 37, wherein the chimeric
2 RSV is a chimera of human RSV A and RSV B which elicits an
3 immune response against both human RSV A and RSV B.

1 44. The method of claim 37, wherein the chimeric
2 RSV is a chimera of human RSV A and RSV B which elicits an
3 immune response against either human RSV A or RSV B and is co-
4 administered with an immunologically sufficient amount of a
5 second attenuated RSV capable of eliciting an immune response
6 against human RSV A or RSV B, whereby an immune response is
7 elicited against both human RSV A or RSV B.

1 45. The method of claim 44, wherein the chimeric
2 RSV and second attenuated RSV are administered simultaneously
3 as a mixture.

1 46. An immunogenic composition to elicit an immune
2 response against RSV comprising an immunologically sufficient
3 amount of the chimeric RSV of claim 1 in a physiologically
4 acceptable carrier.

1 47. The immunogenic composition of claim 46,
2 formulated in a dose of 10^3 to 10^6 PFU.

1 48. The immunogenic composition of claim 46,
2 formulated for administration to the upper respiratory tract
3 by spray, droplet or aerosol.

1 49. The immunogenic composition of claim 46,
2 wherein the chimeric RSV is a chimera of human RSV A and RSV B
3 which elicits an immune response against either human RSV A or
4 RSV B.

1 50. The immunogenic composition of claim 46,
2 wherein the chimeric RSV is a chimera of human RSV A and RSV B
3 which elicits an immune response against both human RSV A and
4 RSV B.

1 51. The immunogenic composition of claim 46,
2 wherein the chimeric RSV is a chimera of human RSV A and RSV B
3 which elicits an immune response against either human RSV A or
4 RSV B and wherein the composition further comprises an
5 immunologically sufficient amount of a second attenuated RSV
6 capable of eliciting an immune response against human RSV A or
7 RSV B, whereby the composition elicits an immune response
8 against both human RSV A or RSV B.

1 52. An isolated polynucleotide molecule comprising
2 a chimeric RSV genome or antigenome which includes a partial
3 or complete RSV genome or antigenome of one RSV strain or
4 subgroup virus combined with a heterologous gene or gene
5 segment of a different RSV strain or subgroup virus.

1 53. The isolated polynucleotide molecule of claim
2 52, wherein the chimeric genome or antigenome comprises a
3 partial or complete human RSV genome or antigenome of one RSV
4 subgroup or strain combined with a heterologous gene or gene
5 segment from a different, human or non-human RSV subgroup or
6 strain.

1 54. The isolated polynucleotide molecule of claim
2 52, wherein the heterologous gene or gene segment is from a
3 human RSV subgroup A, human RSV subgroup B, bovine RSV, avian
4 RSV, or murine RSV.

1 55. The isolated polynucleotide molecule of claim
2 52, wherein the heterologous gene or gene segment encodes a
3 RSV F, G or SH glycoprotein or a cytoplasmic domain,
4 transmembrane domain, ectodomain or immunogenic epitope
5 thereof.

1 56. The isolated polynucleotide molecule of claim
2 52, wherein the chimeric genome or antigenome comprises a
3 partial or complete human RSV A subgroup genome or antigenome

4 combined with a heterologous gene or gene segment from a human
5 RSV B subgroup virus.

1 57. The isolated polynucleotide molecule of claim
2 52, wherein one or both human RSV B subgroup glycoprotein
3 genes F and G is substituted to replace one or both
4 counterpart F and G glycoprotein genes in the RSV A genome or
5 antigenome.

1 58. The isolated polynucleotide molecule of claim
2 57, wherein both human RSV B subgroup glycoprotein genes F and
3 G are substituted to replace the counterpart F and G
4 glycoprotein genes in the RSV A genome or antigenome.

1 59. The isolated polynucleotide molecule of claim
2 52, wherein the chimeric genome or antigenome is further
3 modified by one or more attenuating mutations.

1 60. The isolated polynucleotide molecule of claim
2 52, wherein both human RSV B subgroup glycoprotein genes F and
3 G are substituted to replace counterpart F and G glycoprotein
4 genes within an RSV A genome or antigenome to form the
5 chimeric genome or antigenome, which is further modified to
6 incorporate attenuating point mutations selected from (i) a
7 panel of mutations specifying temperature-sensitive amino acid
8 substitutions Gln₈₃₁ to Leu and Tyr₁₃₂₁ to Asn in the RSV
9 polymerase gene L; (ii) a temperature-sensitive nucleotide
10 substitution in the gene-start sequence of gene M2; (iii) an
11 attenuating panel of mutations adopted from cold-passaged RSV
12 specifying amino acid substitutions Val₂₆₇ Ile in the RSV N
13 gene, and Cys₃₁₉ to Tyr and His₁₆₉₀ Tyr in the RSV polymerase
14 gene L; or (iv) a deletion of the SH gene.

1 61. The isolated polynucleotide molecule of claim
2 52, further comprising a nucleotide modification specifying a
3 phenotypic change selected from a change in growth
4 characteristics, attenuation, temperature-sensitivity,

5 cold-adaptation, plaque size, host-range restriction, or a
6 change in immunogenicity.

1 62. The isolated polynucleotide molecule of claim
2 61, wherein a SH, NS1, NS2, M2ORF2, or G gene is modified.

1 63. The isolated polynucleotide molecule of claim
2 61, wherein the nucleotide modification comprises a nucleotide
3 deletion, insertion, addition or rearrangement of a cis-acting
4 regulatory sequence of a selected RSV gene within the chimeric
5 RSV genome or antigenome.

1 64. A method for producing an infectious attenuated
2 chimeric RSV particle from one or more isolated polynucleotide
3 molecules encoding said RSV, comprising:
4 expressing in a cell or cell-free lysate an
5 expression vector comprising an isolated polynucleotide
6 comprising a chimeric RSV genome or antigenome and RSV N, P, L
7 and RNA polymerase elongation factor proteins.

1 65. The method of claim 64, wherein the chimeric
2 RSV genome or antigenome and the N, P, L and RNA polymerase
3 elongation factor proteins are expressed by two or more
4 different expression vectors.